academicJournals

Vol. 7(25), pp. 3217-3221, 18 June, 2013
DOI: 10.5897/AJMR12.783
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Effect of different pH values on growth and sporulation of *Estye vermicola*

Zhen Wang[1,2], Chun Yan Wang[2], Yun Bo Wang[2], Jian Jie Xue[2], Zheng Li[2], Jing Jie Li[2], Li Guan Gu[2] and Chang Keun Sung[2]*

[1]Engineering Technology Research Center of Southern Shandong Province for TCM Resource Development, Linyi University, Linyi, 276000, People's Republic of China.
[2]Department of Food Science and Technology, College of Agriculture and Biotechnology, Chungnam National University, Daejeon, 305-764, South Korea.

Accepted 17 June, 2013

***Esteya vermicola*, an endoparasitic fungus of pinewood nematode, exhibits great potential as a biological agent against pinewood nematodes. In order to improve the growth rate, sporulation and proportion of lunate conidia, the optimization of pH value for *E. vermicola* was tested. The diameter and dry mass of *E. vermicola* in PDA medium were increased greatly at pH 5 after 15 days, while the diameter and dry mass of *E. vermicola* were inhibited at pH 9 and 10. The sporulation of *E. vermicola* in PDA or PDB medium was highest at pH 6, 2.0 × 10^7 and 1.4 × 10^7 CFU/ml, respectively. The proportion of lunate conidia in PDA medium was highest at pH 4, while the proportion of lunate conidia in PDB medium was highest at pH 7.**

Key words: pH value, *Esteya vermicola*, growth, sporulation.

INTRODUCTION

Esteya vermicola, an endoparasitic fungus of pinewood nematode, exhibits great potential as a biological agent against pinewood nematode. It produces two kinds of conidia, bacilloid and lunate conidia. Bacilloid conidia do not adhere well to nematodes whereas lunate conidia can adhere and infect nematodes by cuticle penetration, immobilization and digestion of the internal contents (Liou et al., 1999). *E. vermicola* showed good results in conptrolling the pinewood nematode by inoculating conidia suspensions into red pine tree (*pinus densiflora*) logs which were killed by pinewood nematodes, or spraying conidial suspensions onto pine seedlings under greenhouse conditions (Wang et al., 2010). The conidia were applied under the condition of different pH values, so the range of pH values for *E. vermicola* growing and the optimization of pH value for mass production should be considered.

It was reported that a medium having pH values between 5 and 6 at the time of inoculation is suitable for most fungi, because fungi generally tolerate more acid than alkali (Lilly and Barnett, 1951). *Colletotrichum gloeosporioides* isolates grow well at pH 5 while sporulation is better at pH 6 (Kumara and Rawal, 2008). Similar observations are also reported by some other authors with different species of *Colletotrichum* (Ramakrishnan, 1941; Sood, 2011). Mycelia of *Coniothyrium minitans* at pH 6 have a higher productivity of conidia production than those grown at pH 4 (Shi, 2008). For *Ceratocystis paradoxa*, the PDA recorded highest diameter at pH 6.5 whereas sporulation intensity was highest at pH 7.0 (Yadahalli, 2007).

Although studies on the effect of pH value on sporulation of fungi have been established, the functions of optimization of pH value for fungi growth, sporulation,

*Corresponding author. E-mail: kchsung@cnu.ac.kr. Tel: +82-42-821-6722. Fax: 82-42-822-2287.

Table 1. Effect of different pH values on diameter and dry mass of *E. vermicola* CNU 120806 in potato dextrose agar medium and potato dextrose broth medium.

pH value	Solid culture		Liquid culture
	Diameter (cm)	Dry mass (g)	Dry mass (g)
4	4.88±0.16^{b}	0.12±0.01^{bc}	0.31±0.03^{b}
5	6.74±0.08^{d}	0.16±0.02^{c}	0.67±0.03^{c}
6	6.43±0.09^{cd}	0.15±0.02^{bc}	0.47±0.01^{bc}
7	6.20±0.24^{c}	0.14±0.00^{bc}	0.41±0.02^{b}
8	4.75±0.30^{b}	0.10±0.01^{abc}	0.04±0.02^{a}
9	4.38±0.40^{b}	0.06±0.01^{ab}	0.01±0.00^{a}
10	2.95±0.43^{a}	0.03±0.01^{a}	

Values are the mean ± standard deviations calculated from three replications. Letters within the same column indicate values that are significantly different at P<0.05 for Turkey's test.

especially the proportion of lunate conidia of *E. vermicola* are largely unknown. The aim of this study was to investigate the optimization of pH value for fungi growth, sporulation and proportion of lunate conidia of *E. vermicola*.

MATERIALS AND METHODS

Fungal strain

E. vermicola CNU 120806 was obtained from the Agriculture Bioscience Biotech Center, Chungnam National University, Korea. It was maintained on potato dextrose agar (PDA) (Acumedia, USA) slants at 4°C and cultured on PDA plates at 26°C.

Culture media

A 4.8 g of potato dextrose broth powder was added into a flask with 200 ml distilled water and was mixed by a glass rod. The pH value of the PDB solution was adjusted to 4, 5, 6, 7, 8, 9 and 10 with HCl solution (11.7%) and NaOH solution (5 M/L). For solid medium, agar powder was added at the level of 3.5% to the PDB solution at pH 2, and at the level of 3% to the PDB solution at pH 3 and 4, and at the level of 2% at pH 5, 6, 7, 8, 9 and 10. For liquid culture medium, no agar was added to the PDB broth. After PDA medium were autoclaved, the PDA medium was poured into Petri dishes.

Cellophane was cut to similar size of Petri dish, transferred into flask with water and autoclaved. In order to separate fungal colony from culture medium, the sterile cellophane discs were placed onto the PDA medium in Petri dishes before inoculation. A 5-mm fungal disc was taken from the periphery of 7-day-culture *E. vermicola* colony and transferred to the center of the PDA medium in each Petri dish, and cultured in an incubator for 15 days at 26°C. For liquid medium, 3 discs from the edge of 7-day-old pure culture (1 cm in diameter) were added to the PDB broth and cultured for 7 days at 26°C.

Growth rate, sporulation and proportion of lunate conidia

The growth rate of *E. vermicola* in PDB medium in different pH values was evaluated after 7 days, and the growth state in PDA medium in different pH values was evaluated after 15 days. For PDB medium in different pH values, the mycelium in liquid culture medium was filtrated by filter paper and was dried in oven at 60°C for 2 days. The suspension of PDB medium was oscillated using an oscillator (Vortex genie, USA) at a speed of 600 oscillations per minute with 0.5% (v/v) Tween 80 (Sumchun, Korea) and several glass beads. Concentration of conidia was determined using a hemocytometer (Marienfeld, Germany). The size of two conidia which were produced from *E. vermicola* was quite different. One was lunate which can adhere, infect and kill nematode, the other was bacilloid. The lunate conidia were counted and the proportion of lunate conidia was measured.

Diameters of colonies were measured in PDA medium in different pH values. Some colonies which were cultured in PDA medium were isolated from cellophane and transferred to an oven at 60°C for 2 days. Furthermore, other colonies which were cultured in PDA medium were transferred into 50 ml tubes with 0.5% (v/v) Tween 80 and several glass beads. To dislodge conidia from conidiophores, the tubes were oscillated using an oscillator at a speed of 600 oscillations per minute. Concentration of conidia was determined using a hemocytometer. The lunate conidia were counted and the proportion of lunate conidia was measured.

Data analysis

All experiments were conducted in three replications and repeated 3 times, and the data were presented as means and standard deviations. The effects of pH value on growth, sporulation and proportion of lunate conidia were compared and analyzed statistically. The significance of differences was determined by one-way analysis of variance (ANOVA) with Tukey's multiple comparison module of the Minitab statistical software, version 13.30 (Minitab Inc., State College, PA, USA) and differences with *P* values of <0.05 were considered statistically significant.

RESULTS AND DISCUSSIONS

Effects of different pH values on growth rate

Effects of different pH values on diameter and dry mass of *E. vermicola* in PDA and PDB medium are shown in Table 1. The pH range of *E. vermicola* for growth varied between 4 and 10 in PDA medium, and varied between 4 and 9 in PDB medium. The diameter of *E. vermicola* in PDA medium was increased greatly at pH 5, 6 and 7 after 15 days, while the diameter of *E. vermicola* was inhibited greatly at pH 10. Effects of different pH values on diameter of *E. vermicola* in PDA medium showed positive correlation to dry mass. Dry mass was increased greatly in PDA medium at pH 5 and 6, 4-fold more than that at pH 10. In PDB medium, the dry mass was enhanced at pH 5 and 6 after 7 days, while the growth of dry mass was inhibited greatly at pH 9 and 10.

E. vermicola grew better in weak acid environment than in weak alkaline environment. Moreover, *E. vermicola* could not grow in PDA medium below pH 3 and above pH 11, and it could not grow below pH 3 and above pH 10 in PDB medium. That may be because the strong acid or strong base destroyed the DNA of chromosome and protein, and inhibited conidia growth.

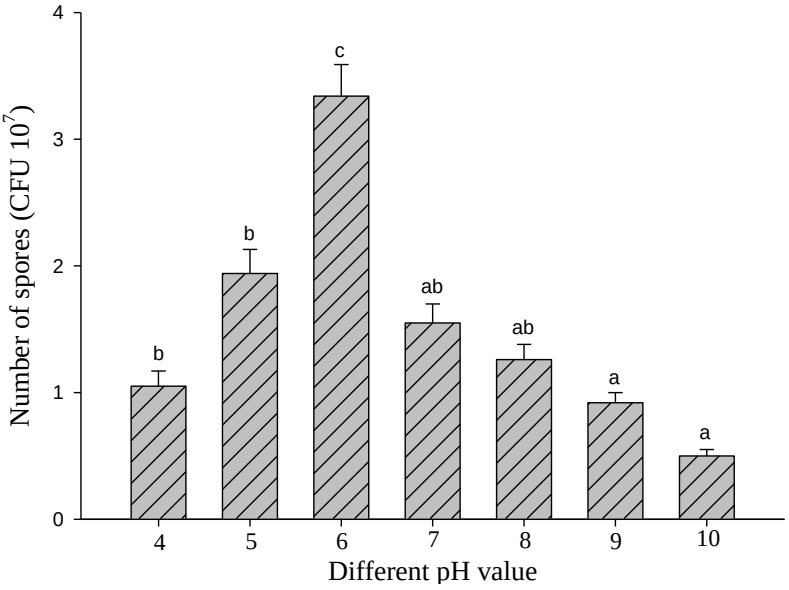

Figure 1. Effect of different pH values on sporulation of *E. vermicola* CNU 120806 in potato dextrose agar medium cultured at 26°C for 15 days. Letters above similar bars indicate significant differences at P<0.05 for Turkey's test.

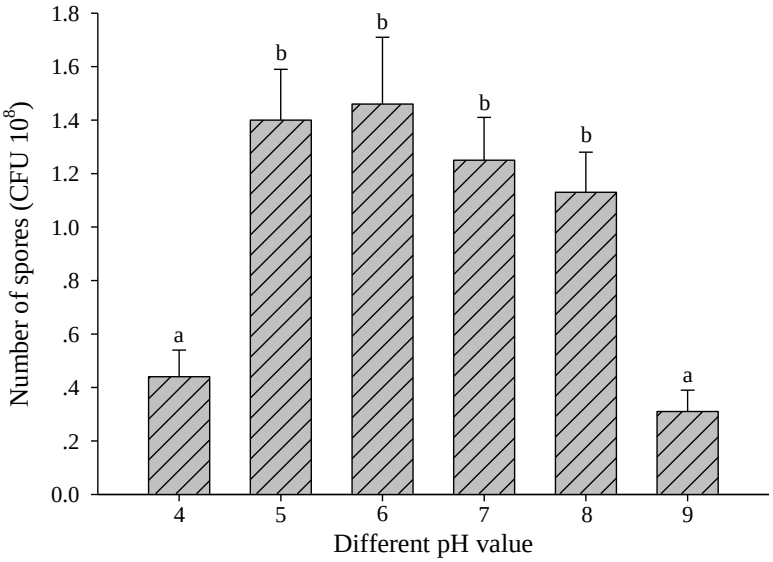

Figure 2. Effect of different pH values on sporulation of *E. vermicola* CNU 120806 in potato dextrose broth medium cultured at 26°C for 15 days. Letters above similar bars indicate significant differences at P<0.05 for Turkey's test.

Effects of different pH values on sporulation

Effect of different pH values on sporulation of *E. vermicola* in PDA medium is shown in Figure 1. The sporulation of *E. vermicola* was enhanced greatly at pH 6, 3.3×10^7 CFU, 3-fold more than that at pH 9 and 10. The sporulation was inhibited gradually above pH 7. Therefore, *E. vermicola* produced more conidia in weak acid environment than that in weak alkaline environment. Effect of different pH values on sporulation of *E. vermicola* in PDB medium is shown in Figure 2. The sporulation of *E. vermicola* was enhanced greatly at pH 5 and 6 in PDB medium, while the sporulation was inhibited gradually above pH 7.

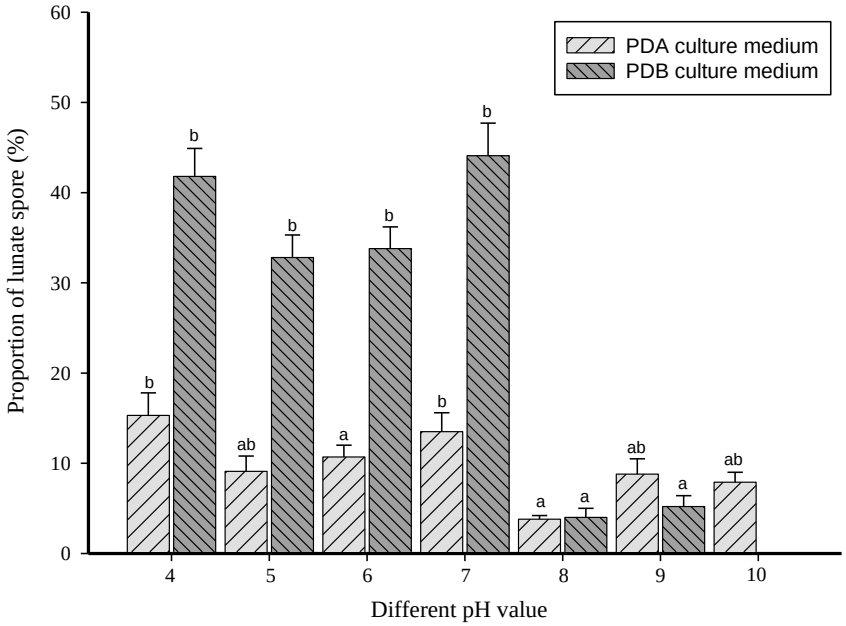

Figure 3. Effect of different pH values on proportion of lunate conidia of *E. vermicola* CNU 120806 in potato dextrose agar and potato dextrose broth medium. Letters above similar bars indicate significant differences at P<0.05 for Turkey's test.

The growth rate was highest at pH 5 in PDA or PDB medium, while the sporulation was highest at pH 6 in PDA or PDB medium. That may be because *E. vermicola* could grow more quickly and produce more mycelia at pH 5 than that at pH 6, however conidiophores could produce more lunate and bacilloid conidia at pH 6 than that at pH 5. Sood (2011) got the similar result that growth of *Aspergilli umbrosus* in Asthana and Hawker's, and Currie's medium was optimum at pH 5 while sporulation and secondary metabolite production were moderate.

Effects of different pH values on proportion of lunate conidia

Effects of different pH values on the proportion of lunate conidia in PDA or PDB medium are shown in Figure 3. The proportions of lunate conidia in PDA or in PDB medium were higher at pH range between 4 and 7 than that at pH range between 8 and 10. The proportion of lunate conidia in PDA medium was highest at pH 4, 4-fold higher than that at pH 8. Moreover, the proportion of lunate conidia in PDB medium was highest at pH 7, 11-fold higher than that at pH 8.

In this study, the proportion of lunate conidia in PDB medium was higher than in PDA medium. It was reported that *in vitro* on enriched media, the hyphae produced primarily the cylindrical, elongate, subulate multiseptate, simple or branched conidiophores and conidiogenous cells bearing cylindrical to bacilloid, not lunate conidia of *E. vermicola* (Liou, 1999). The solid PDA medium contains more nutrition than the liquid PDB medium. Moreover, it can provide more oxygen for fungal growth. The PDA medium produced less lunate conidia, therefore, it had lower proportion of lunate conidia than that in PDB medium.

In conclusion, the growth rate, sporulation and proportion of lunate conidia in PDA and PDB medium were increased greatly in pH values between 5 and 7. The growth rate was highest at pH 5 in PDA and PDB medium, and the sporulation of *E. vermicola* was highest at pH 6 in PDA and PDB medium. The proportion of lunate conidia in PDA medium was highest at pH 4, and the proportion of lunate conidia in PDB medium was highest at pH 7. This study may provide proper pH value for mass culture and application of *E. vermicola* to control pine wilt disease.

REFERENCES

Kumara KLW, Rawal RD (2008). Influence of carbon, nitrogen, temperature and PH on the growth and sporulation of some Indian isolates of *Colletotrichum gloeosporioides* causing anthracnose disease of papaya (Carrica papaya L). Trop. Agr. Res. Ext. 11: 7-12.

Lilly VG, Barnett HL (1951). Physiology of Fungi. McGraw Hill Book Company Inc., New York. pp. 464.

Liou JY, Shih JY, Tzean SS (1999). *Esteya*, a new nematophagous genus from Taiwan, attacking the pinewood nematode (*Bursaphelenchus xylophilus*). Mycol. Res. 103: 242–248.

Ramakrishnan TS (1941). Studies in the genus *Colletotrichum*. II. Physiological studies on *Colletotrichum falcatum* Went. Proc. Indian Acad. Sci. 14(4): 395-411.

Shi JL, Li Y, Zheng Y, Zhu Y, Zhang XP, Du GC, Chen J (2008). Tryptophan supplementation and pH adjustment for optimizing the sporulation of *Coniothyrium minitans*. Biotechnol. Lett. 30: 259–262.

Sood M (2011). Cultural Physiology: Effect of culture mediums and pH on the growth, sporulation and secondary metabolites production of *Aspergillus Umbrosus*. J. Ecobiotechnol. 3(5): 8-11.

Wang CY, Fang ZM, Wang Z, Zhang DL et al. (2010). Biological control of the pinewood nematode *Bursaphelenchus xylophilus* by application of the endoparasitic fungus *Esteya vermicola*. Biocontrol 56:91-100.

Yadahalli KB, Adiver SS, Kulkarni S (2007). Effect of pH, temperature and relative humidity on growth and development of *Ceratocystis paradoxa* – a causal organism of pineapple disease of sugarcane. Karnataka J. Agric. Sci. 20(1):159-161.